

## The skin microbiota in equine pastern dermatitis: a case-control study of horses in Switzerland

Sarah Kaiser-Thom\*, Markus Hilty†, Shannon Axiak‡ and Vinzenz Gerber\*

\*Swiss Institute of Equine Medicine (ISME), Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, and Agroscope, Länggassstrasse 124, 3012 Bern, Switzerland

†Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, 3010 Bern, Switzerland

‡Clinical Anaesthesiology, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Länggassstrasse 124, 3012 Bern, Switzerland

Correspondence: Sarah Kaiser-Thom, Swiss Institute of Equine Medicine (ISME), Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, and Agroscope, Länggassstrasse 124, 3012 Bern, Switzerland. E-mail: s.kaiserthom@gmail.com

**Background** – Equine pastern dermatitis (EPD), a multifactorial syndrome, manifests as skin lesions of variable severity in the pastern area. Despite the widespread use of antibacterial therapy for treating this condition, little is known about the contributing bacteria.

**Hypothesis/Objectives** – To investigate the bacterial skin microbiota in EPD-affected and unaffected (control) pasterns.

**Animals** – Case-control study with 80 client-owned horses; each with at least one EPD-affected and one control pastern.

**Methods and materials** – Horses were grouped by the form of EPD (mild, exudative or proliferative), the assigned severity grade and type of pretreatment (disinfectant, topical antibacterial or no antibacterial pretreatment). Skin swabs were obtained, and the microbiota composition was compared between the groups.

**Results** – Bacterial alpha diversity was reduced in affected pasterns ( $P < 0.001$ ) and this reduction was significantly associated with the EPD forms ( $P < 0.001$ ), and not with the type of pretreatment ( $P > 0.14$ ). Analyses of beta-diversity confirmed a disordering of the skin microbiota ( $P = 0.004$ ) in affected versus control pasterns, that was particularly profound in more severe lesions. The type of pretreatment was not significantly associated with this disordering. Four differentially abundant families were detected, of which Staphylococcaceae was the most distinct. The relative abundance of staphylococci was significantly increased in affected pasterns ( $P = 0.011$ ), particularly in those that had received antibacterial treatment previously.

**Conclusions and clinical relevance** – Changes in the microbiota are associated with the EPD form or severity of lesions. The role of bacteria in the pathogenesis of EPD as well as the propriety and consequences of antibacterial treatment should therefore be further investigated.

### Introduction

Equine pastern dermatitis (EPD) is a multifactorial syndrome that has been documented for over 200 years<sup>1</sup> and still represents a common clinical problem in equine medicine. Clinical manifestations are characterized by skin lesions located in the caudal aspect of the pasterns, and when severe, can compromise a horse's wellbeing. Despite the high prevalence of EPD,<sup>2,3</sup> its pathogenesis remains poorly understood. Several intrinsic and extrinsic factors contributing to the development and persistence of EPD lesions have been proposed. Besides genetic predispositions<sup>2,4</sup> and environmental conditions,<sup>5–9</sup> parasitic, fungal and bacterial pathogens may play a role.<sup>7–12</sup>

Recently, culture-independent assays have enabled a more inclusive, yet complex view of the role bacteria play in normal skin colonization and in wound-healing. These assays offer a more comprehensive picture of the dermal microbiota and have helped to develop robust evidence that bacteria influence cutaneous inflammations in several ways.<sup>13–15</sup> As an external covering of the body, the skin with its normal microbiota serves as the "critical first line of defence" against foreign pathogens,<sup>16</sup> and even in intact skin there is a constant balancing act between tolerating and repelling colonizing bacteria.<sup>17</sup> Following skin barrier disruption, wound-healing normally proceeds in three phases: inflammation, tissue formation and tissue remodelling.<sup>18</sup> In all three phases, contact of bacterial populations – both commensal or pathogenic – with subcutaneous tissue can perpetuate and amplify the immune response and resulting inflammation,<sup>19,20</sup> with the potential to develop chronic wounds that are stalled in a cycle of persistent inflammation. Such persistent inflammation is observed regularly in EPD, yet its association with alterations in the local microbiota has not been investigated. Because EPD is frequently a recurrent condition, horses

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often are treated with various different products and classes of pharmacological and physical agents and therapies.<sup>7,8,21,22</sup>

In the present study we aimed to examine the microbiota in EPD-affected and unaffected control pasterns using 16S rRNA gene sequencing. Bacterial alpha and beta diversity indices and their relationships with previously defined clinical forms and severity grades were analysed. We also attempted to explore if different types of pretreatments, specifically those with antibacterial properties, additionally influenced the microbiota in affected pasterns.

## Methods and materials

### Ethics

The study protocol was approved by the Veterinary Ethical Committees of all 26 cantons in Switzerland (VD3297+). The participants were recruited mainly through announcements on social media platforms (see details in Appendix S1 in Supporting information). Written informed consent was obtained from all horse owners before examination and sampling.

### Study design

The project was designed as a case-control study with cross-sectional sampling. All horses underwent a general physical examination, followed by thorough inspection of all four pasterns. When indicated, the pastern hair was trimmed. Diagnosis of EPD was based on evidence of clinical signs including scales, crusts, ulceration and formation of skin folds.<sup>7–9</sup>

In order to compare microbiota from EPD-affected and unaffected pasterns, horses were enrolled if they met the following two inclusion criteria: First, they needed to have one affected and at least one control-suitable pastern. If a horse had several control-suitable pasterns, samples were taken from two (controls 1 and 2). Then, all pasterns were graded for lesion severity using a standardized scoring system in order to objectively select the worst-affected pastern (see details in Appendix S1). The cumulative score could range from 0 points (unaffected) to 21 points (severely affected), and pasterns that scored  $\geq 4$  were classified as EPD-affected. Pasterns were identified as controls if their score was  $\leq 3$ . This cut-off was chosen based on the observation that even unspecific and mild clinical signs, like slight erythema, resulted in a score of  $>0$ . The second inclusion criterion was that the affected pastern and its respective control pastern had to differ by  $\geq 5$  scoring points to ensure good contrast between groups. Furthermore, affected pasterns also were classified into one of the three EPD forms (mild, exudative or proliferative) as defined previously.<sup>7</sup> The owners completed a questionnaire on the horse's management and medical history. Horses that had received antimicrobial drugs systemically in the six months before sample collection were excluded. Accounts of topically applied treatments for EPD within the last six months were recorded, and subsequently categorized into three groups (disinfectant, antibacterial or no antibacterial pretreatment). Owners were asked to show documentation of all medication to the investigator for verification.

### Sample collection

The most severely affected pastern and at least one control pastern were sampled. Samples from additional control pasterns were collected when available. An area of approximately 4 x 2 cm was gently swabbed using sterile cotton swabs (PS/Viscose, Sarstedt AG & Co.; Nümbrecht, Germany) moistened with sterile 0.9% saline solution. A negative control sample was taken for each visit by exposing a moistened swab to the ambient air for 20 s. Swabs were transported in a cold-storage box and subsequently stored at  $-80^{\circ}\text{C}$  until further processing.

### Sample preparation for sequencing analyses

The QIAmp DNA Mini Kit (Qiagen; Hilden, Germany) was utilized for DNA extraction according to the manufacturer's recommendations. From these extracts, the V4 region of the bacterial 16S rRNA gene was amplified using previously described forward (5'-GTGCCAGCMGCCGCGGTAA-3') and reverse (5'-GGACTACHVGGGTWTCTAAT-3') primers.<sup>23</sup> The 16S rRNA PCR was run as described.<sup>24</sup> Cycling conditions were: initial denaturation at  $95^{\circ}\text{C}$  for 6 min; 35 cycles of denaturation at  $95^{\circ}\text{C}$  for 30 s; annealing at  $59^{\circ}\text{C}$  for 30 s; elongation at  $72^{\circ}\text{C}$  for 1.5 min; and a final elongation step at  $72^{\circ}\text{C}$  for 5 min. After purification, PCR products were quantified to confirm a minimum concentration of 1 ng/ $\mu\text{L}$  and samples above this threshold were submitted to the next-generation sequencing platform at the Institute of Genetics, University of Bern for indexing and paired-end 2 x 250 bp sequencing on the Illumina MiSeq platform (Illumina Inc.; San Diego, CA, USA).

### Raw sequencing data processing

Raw sequencing data were processed using the DADA2 pipeline within the open-source software R,<sup>25</sup> according to the package builder's recommended workflow.<sup>26</sup> Forward reads were trimmed at 240 bp and reverse reads were trimmed at 220 bp, whilst ensuring a Phred quality score of  $\geq 30$  at any point up to the trimming borders, which corresponds to a base call accuracy of 99.9%. Reads then were denoised by modelling and correcting amplicon errors using the DADA2 algorithm with default parameters. Sequences containing ambiguous base calls, as well as sequences with inappropriate length ( $<245$  bp and  $>257$  bp), and chimeras were identified and removed. Finally, taxonomy was assigned to the remaining amplicon sequence variants (ASVs) by utilizing the Silva 16S rRNA reference database v132<sup>27</sup> and sequences identified as chloroplasts, mitochondria, Archaea or Eukaryotes were erased.

In horses where two control samples had been taken, R's *sample* function was used retrospectively to select by random choice which of the two controls in a horse was designated Control 1 and Control 2. If not stated otherwise, the following comparisons were made between Control 1 and affected.

### Statistical and microbiota analyses

The chi-squared test was used to compare groups. Microbiota data were analysed in R using the BASE, VEGAN, MICROBIOME, LME4 and PAIRWISEADONIS packages, and plots were created with the BASE, GGPlot2, GGPUBR and SJPlot packages. Rarefaction curves were generated to affirm that sequencing depth was sufficient.

For the evaluation of the within-sample diversity, bacterial richness (number of ASVs), evenness and the Shannon index were calculated. Univariate Wilcoxon signed-rank tests were used to compare these measurements between affected and control pasterns. Multilevel mixed-effects models then were fitted in order to determine the influence of the EPD form or score group as well as the pretreatment on the alpha diversity measurements. The horse ID was included as a random factor to account for the paired samples.

The dissimilarity of bacterial composition between samples (beta diversity) was quantified by means of weighted (abundance-based) Bray–Curtis indices, which were compiled into a distance matrix. Analyses of the unweighted (presence/absence-based) Bray–Curtis indices can be found in Appendix S1. A permutational multivariate ANOVA (PERMANOVA, function *adonis* as part of the R/VEGAN package) was performed using the distance matrix and both the EPD form and the pretreatment as parameters, followed by *post hoc* testing for multilevel pairwise comparisons. Subsequently, a Procrustes analysis was conducted to quantify the pairwise difference in microbiota composition between the affected pastern and its control. For this, nonmetric multidimensional scaling (NMDS) was performed separately on weighted distance matrices of the affected pasterns and the controls, resulting in two ordinations which were then compared (functions *procrustes* and *protest*). Group differences of Procrustes residuals exceeding the upper quartile were examined using a chi-square test of independence.

Barplots were created to visualize the bacterial compositions in the samples grouped by the form of EPD and the type of pretreatment. Relative abundances of the 10 most abundant families were compared between affected and control pasterns using univariate Wilcoxon signed-rank tests with Bonferroni corrections.

Furthermore, the intra- and interindividual differences in the beta diversity of samples were analysed. Only controls were used for this specific evaluation, comparing the mean dissimilarity distances of pasterns within the same horse (Control 1 versus Control 2), between horses living in the same stable, and between horses living in different stables. All relevant pairwise distances were compiled from the distance matrices and groups were compared by an unpaired Wilcoxon rank-sum test. A  $P$ -value of  $<0.05$  was considered as statistically significant for all comparisons.

## Results

### Overview of samples

Swabs were taken from a total of 191 pasterns taken from 80 horses of different breeds and ages. Samples included 80 swabs from affected pasterns (35 mild forms, 27 exudative and 18 proliferative forms) and 111 from control pasterns (80 Control 1 and 31 Control 2). The Control 2 samples were used only for one particular analysis at the end: the comparison of intra- versus interindividual differences. Furthermore, based on the awarded severity score, all other 160 samples also were divided into score groups: Group I with scores 0–3 (the Control 1 samples,  $n = 80$ ), Group II with scores 5–8 ( $n = 23$ ), Group III with scores 9–11 ( $n = 34$ ), and Group IV with scores  $>12$  ( $n = 23$ ); with all proliferative and exudative forms being in severity groups III and IV.

Of the 80 horses, 25 had previously received topical antibacterial treatment (including 11 mild EPD cases, 10 exudative and four proliferative); 29 had been treated with disinfectants (including 10 mild EPD cases, 11 exudative and eight proliferative); and 26 had not received antibacterial treatment (including 14 mild EPD cases, six exudative and six proliferative). There was no association between groups of EPD and pretreatment ( $P = 0.51$ ). Further details on the study population can be found in the supplementary material (S3, S4, S5).

### Alpha diversity is decreased in affected samples

A total of 15,270,581 reads were retained in our study with a median of 75,876 reads per sample. Clustering resulted in 47,043 ASVs. Because all rarefaction curves reached their plateau, the sequencing depth was found to be sufficient.

Bacterial richness was not significantly impaired in affected pasterns ( $P = 0.07$ ,  $r = 0.20$ ), whereas species evenness was ( $P < 0.001$ ,  $r = 0.61$ ). Hence, the Shannon diversity index, that accounts for both abundance and evenness of the present species, was significantly reduced in affected pasterns in comparison to their controls ( $P < 0.001$ ,  $r = 0.54$ ). On average, the Shannon diversity amounted to one index point less in affected pasterns. Multilevel mixed-effects models revealed that both species evenness and the Shannon index were associated with the form of EPD ( $P < 0.001$ ), and not with the type of pretreatment ( $P > 0.14$ ). In particular, the exudative form of EPD was linked to a decreased evenness ( $P = 0.001$ ) and Shannon diversity ( $P = 0.001$ ). The

results are visualized in Figure 1. Similar results were obtained when the model was implemented with the samples classified by score groups instead of the EPD form ( $P = 0.001$ , data not shown).

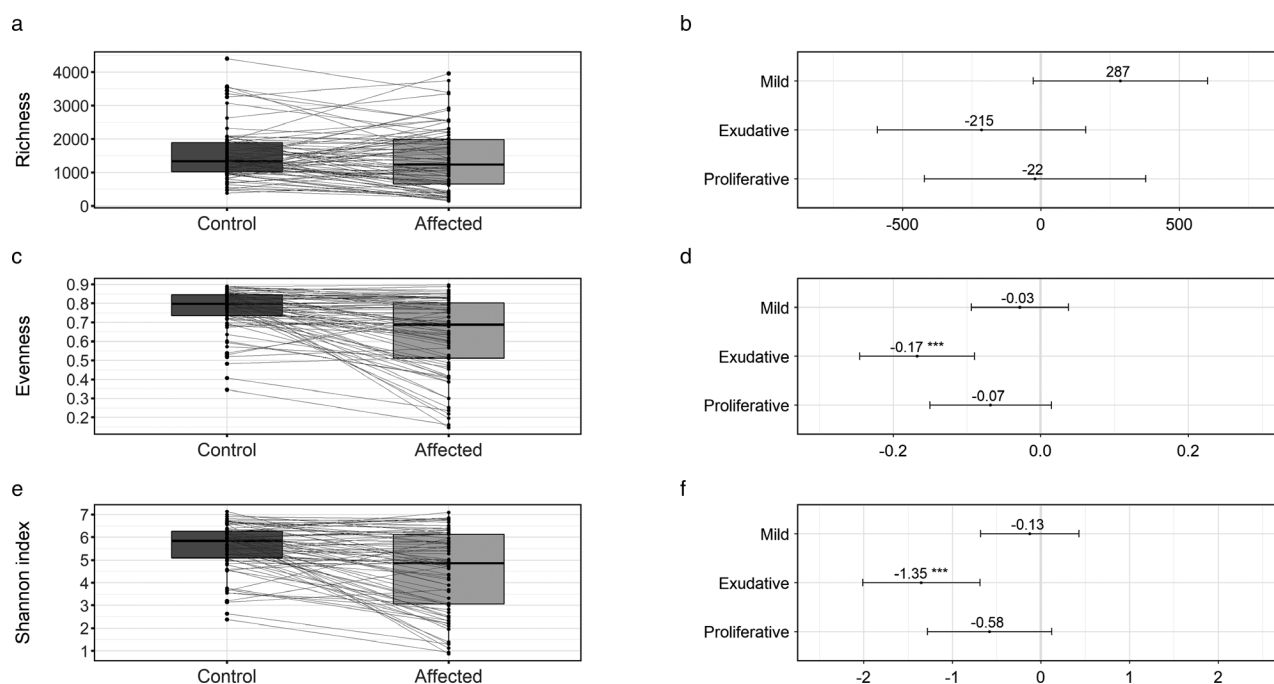
### Microbiota is disordered in exudative and proliferative lesions

After alpha diversity analyses, overall differences in beta diversity relating to the EPD form and the pretreatment were analysed using *adonis*. We found that the control samples were rather closely clustered (Figure 2), and that the bacterial composition in the affected pasterns was altered in relation to both the EPD form ( $P = 0.001$ ) and the pretreatment ( $P = 0.023$ ). Pairwise *post hoc* testing found that pasterns with exudative or proliferative lesions differed significantly from the controls, whereas pasterns with mild EPD did not. Moreover, antibacterial pretreatment also significantly altered the microbiota (Table 1).

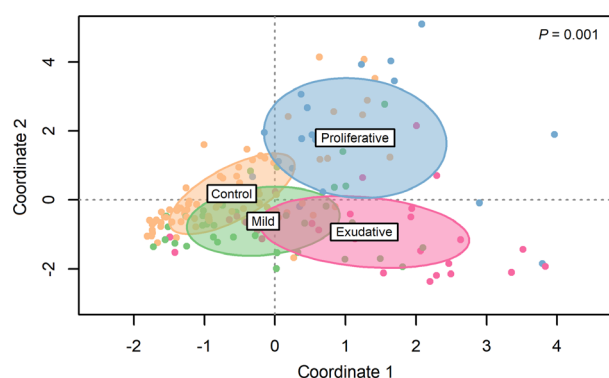
The *adonis* test, however, only allows for comparison of groups and the pairing of samples cannot be accounted for. Therefore, in a next step, pairwise ordination changes of bacterial communities in affected pasterns and their respective controls were quantified. The Procrustes analysis matches two ordinations as close as possible whereby the resulting residuals indicate actual divergence.<sup>28</sup> The analysis revealed that the microbiota was significantly disordered in affected pasterns as compared to controls ( $P = 0.004$ ). Depending on the form, clusters of microbiota were deflected in distinct directions (*adonis* test: adj.  $P = 0.003$ ; Figure 3a). Proliferative and exudative lesions induced larger alterations than mild lesions, with mean residual values of 0.20, 0.18 and 0.14, respectively; however, group differences in the residuals produced by the individual EPD forms were not significant (adj.  $P = 0.690$ ; Figure 3b). The effect of the pretreatment on ordination alterations was only modestly evident (*adonis* test: adj.  $P = 0.078$ ). When samples were grouped by our scoring system, it became apparent that the higher the cumulative score, the greater the microbiota alterations (*adonis* test: adj.  $P = 0.024$ , data not shown), which is very much in line with assigned EPD forms. Mean residual values for samples sorted according to score groups II, III and IV were 0.11, 0.18 and 0.20, respectively, and group differences in residuals here proved to be significant (adj.  $P = 0.049$ ; Figure 3c).

The 10 most abundant families are shown in Figure 4a. In the control pasterns, Moraxellaceae ranked first in average relative abundance, followed closely by other families, so that the overall picture appeared balanced and with a high degree of diversity. In the affected pasterns, four families were found to be differentially abundant after pairwise comparison with their respective control (Figure 4b). Staphylococcaceae were significantly increased in affected pasterns, particularly in those with exudative and proliferative lesions, and in pasterns that had been treated previously with antibacterial agents. The other three differentially abundant families were Sphingomonadaceae, Burkholderiaceae and Microbacteriaceae, and each of these was decreased in relative abundance in the affected pasterns.

The analysis of intra- and interindividual differences in the beta diversity of samples showed that the pastern



**Figure 1.** Paired box plots depict that (a) bacterial richness is only marginally compromised by equine pastern dermatitis (EPD). However, (e) the Shannon diversity is significantly reduced in affected pasterns in that (c) the species are less evenly distributed. Model plots break down the influence of the assigned EPD forms on bacterial richness (b), evenness (d) and the Shannon diversity index (f). The reference group for the models was the control pasterns.



**Figure 2.** Ordination plot for a constrained analysis of proximities (CAP) in relation to the equine pastern dermatitis (EPD) forms. The plots are based on weighted Bray–Curtis dissimilarities. A clustering between the groupings is evident, indicating that pasterns within a group share a certain similarity regarding the bacterial composition on their skin and can be distinguished from other groups. Samples from exudative and proliferative EPD lesions show a greater dissimilarity to the controls than samples from mild EPD lesions.

microbiota within a horse were the most similar to each other in comparison to pasterns from different horses or even more so from different stables (Figure 5).

## Discussion

Using sequence-based methods, we identified alterations of the skin microbiota in EPD-affected pasterns and also observed potential associations with antibacterial pretreatment. In particular, the more severe forms of EPD exhibited profound changes, with a reduction of bacterial alpha diversity in relation to the overgrowth of certain species. Thus, our study offers new insights into the

**Table 1.** *F*- and *P*-values obtained by the *adonis* analysis of beta diversity for the parameters equine pastern dermatitis (EPD) form and pretreatment, and *F*- and adjusted *P*-values obtained by pairwise *post hoc* testing.

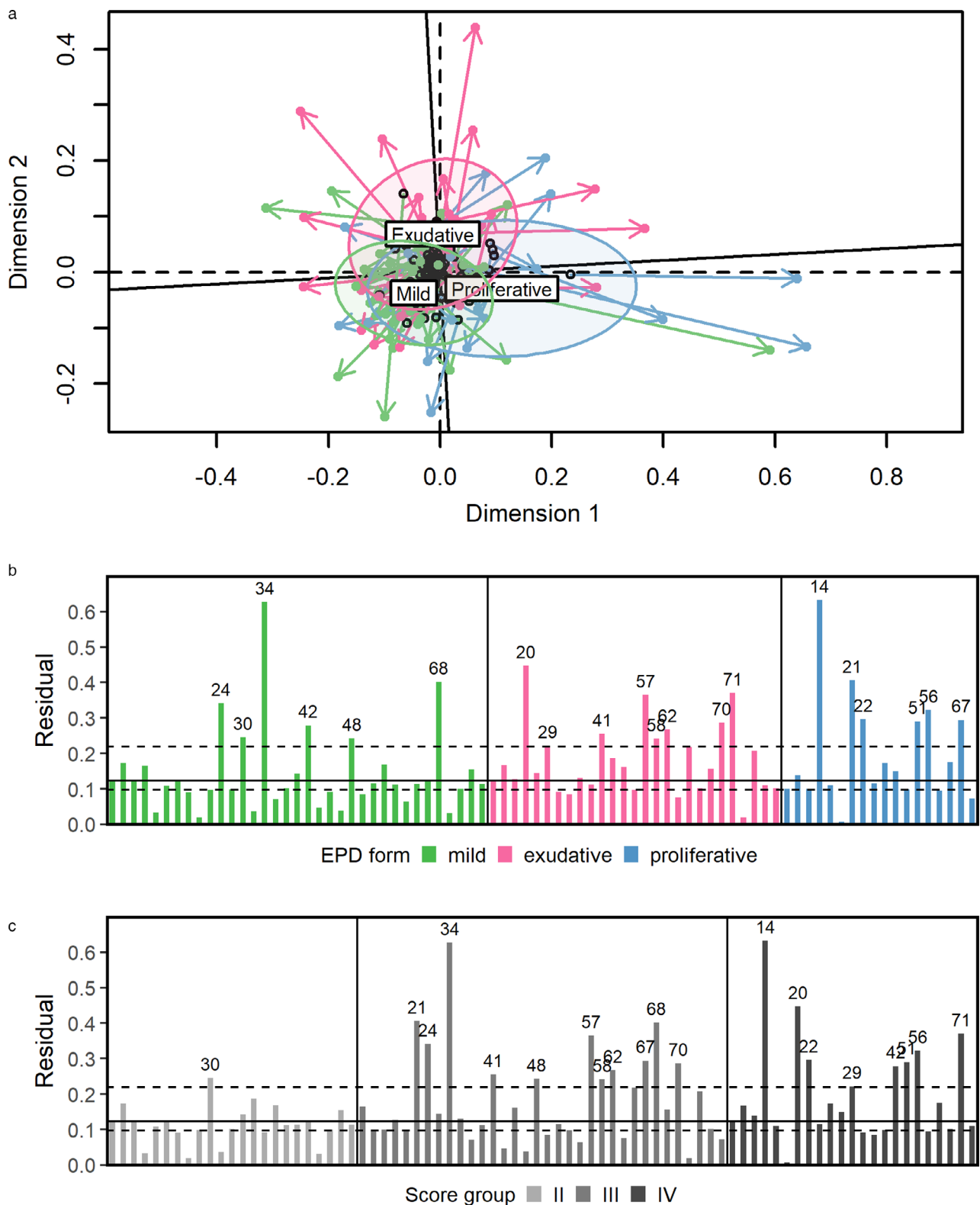
	Parameter	<i>F</i> -value	<i>P</i> -value
Overall <i>adonis</i> analysis	EPD form	2.18	0.001***
	Pretreatment	1.33	0.023*
	Interaction	1.26	0.061
	Comparison	<i>F</i> -value	adj. <i>P</i> -value
Pairwise <i>adonis</i> – EPD forms	Control versus mild	1.14	1.000
	Control versus exudative	3.54	0.004**
	Control versus proliferative	2.69	0.006**
	Mild versus exudative	1.86	0.036*
	Mild versus proliferative	1.87	0.024*
Pairwise <i>adonis</i> – pretreatment	Exudative versus proliferative	1.19	1.000
	No antibacterial pretreatment versus disinfectant	2.97	0.003**
	No antibacterial pretreatment versus antibiotic	1.90	0.012*
	Disinfectant versus antibiotic	1.08	0.957

EPD equine pastern dermatitis.

Levels of significance: \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ .

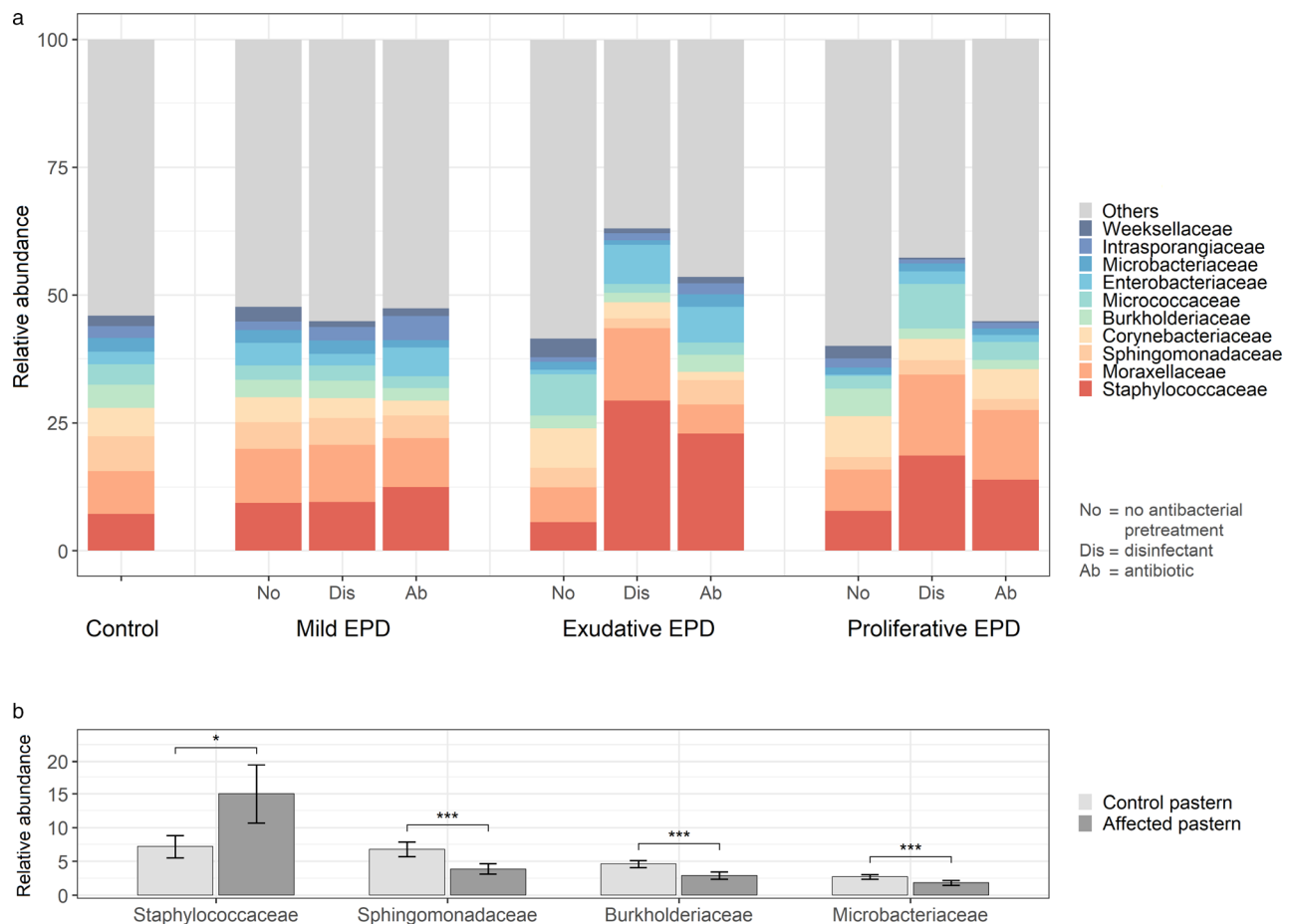
bacteria present in EPD, whose involvement in the disease, although widely assumed,<sup>7–9</sup> has not yet been investigated adequately.

We revealed that bacterial alpha diversity was significantly reduced on sites with visible lesions and this reduction was driven largely by decreased species evenness suggesting that potential pathogens were outcompeting

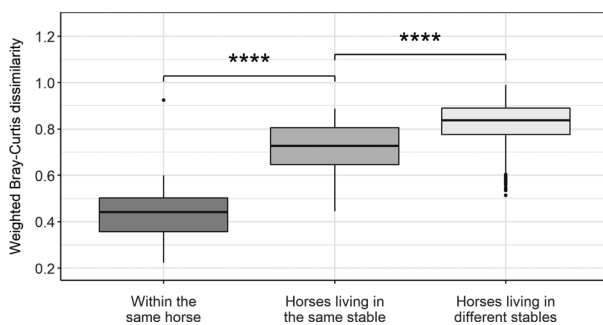


**Figure 3.** Investigation of bacterial community composition using Procrustes analysis, with which two ordinations (one each for the affected and the control pasterns) are compared.

Plots are coloured and ordered by the pastern dermatitis forms (a, b) and the score groups (c). (a) The black circles in the middle represent the control pasterns and show the position of the samples in the first ordination. The arrows point to the position of the respective affected pastern in the target ordination. The length of the arrows corresponds to the residual values. Also, the rotation between the two ordinations is shown, which is necessary to make them match as closely as possible. It becomes evident that, depending on the equine pastern dermatitis (EPD) form, clusters of microbiota are deflected in distinct directions. In the lower two plots (b, c), the residual values of the Procrustes analyses were laid out alongside each other. The higher the residual value, the greater the bacterial dissimilarity between the affected pastern and its control. The numbers denote the horse ID.



**Figure 4.** (a) Stacked bar plots of the 10 most abundant bacterial families. For overview purposes, the remaining 425 detected families with mean relative abundances of <1.9% each were summarized as “others”. The bars were divided according to the form of pastern dermatitis and the pre-treatment. (b) Of these top 10 families, four were found to be differentially abundant after Bonferroni correction of pairwise comparisons of the affected pasterns with the respective control. The mean and the 95% confidence interval are shown. Although Staphylococcaceae are increased in affected pasterns, the other families are decreased in abundance. Levels of significance: \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$



**Figure 5.** Box plots depicting the bacterial dissimilarities between control pasterns.

The analysis of intra- and interindividual differences showed that the pastern microbiota within a horse were more similar to each other in comparison to pasterns from different horses or even more so from different stables, suggesting host specificity of the microbiota as well as a smaller location effect.

or overgrowing other bacteria. A loss of diversity likewise has been reported in a variety of inflammatory skin conditions in domestic animals, such as bovine digital dermatitis and ovine foot rot,<sup>29,30</sup> and in humans, including diabetic ulcers and chronic pressure sores,<sup>31–33</sup> and also is found to be associated with impaired and prolonged

wound healing.<sup>31</sup> The reduction of local diversity in EPD-affected pasterns was further accompanied by a significant disordering of the microbiota, which our beta-diversity analyses found to be related mainly to the different lesion forms and the likely dependent factor of severity. Each of the three EPD forms steered the bacterial composition in a certain direction, with exudative and proliferative lesions causing greater perturbations than mild lesions. By contrast, we found that although previous antibacterial treatment did not act as a primary directing force, it nonetheless likely enhanced the alterations.

The classification of affected pasterns into one of the three forms used herein, as described previously,<sup>7</sup> is certainly helpful for the clinician. However, transitions between the different forms can be blurred. This is why we additionally graded all lesional sites using a cumulative scoring system. The overlap between the mild EPD form and score Group II was large, as were the overlaps between the exudative and the proliferative forms and the two higher score groups; however, these overlaps were not exclusive. For cases in the grey zone, our scoring system offered a slightly better resolution, especially in terms of explanatory or predictive power regarding microbiota perturbations. Therefore, it also may be of use for future studies or in clinical practice.

Of the four differentially abundant families, Staphylococcaceae were the most distinct. This family includes the well-known genus *Staphylococcus* with several of its species being commonly regarded as opportunistic pathogens.<sup>34</sup> Of note, any observed shift here refers to the relative and not quantitative abundance, as for the latter, quantitative PCR methods would be necessary. Nevertheless, it is reasonable to conclude that a bacterial imbalance in favour of staphylococcal species had developed in affected pasterns. Staphylococcal growth is a frequently reported phenomenon following skin barrier disruption not only in humans,<sup>35–37</sup> but also in horses.<sup>38–41</sup> For example, one study detected staphylococci in almost a third of samples from horses with skin disorders of diverse aetiologies.<sup>40</sup> Likewise, a large-scale German survey of wound infection rates in companion animals found “alarming proportions of MRSA [Methicillin-resistant *Staphylococcus aureus*]” and 23% of tested equine samples were positive for *S. aureus*.<sup>41</sup>

Strikingly, the alterations of the microbiota including the shift towards Staphylococcaceae were exceptionally pronounced in exudative and proliferative EPD lesions that previously had received antibacterial treatment. This is of particular clinical interest because these types of lesions justifiably induce owners and veterinarians to initially apply disinfectants or antimicrobial therapy,<sup>7,8,42</sup> the latter in most cases without a bacteriological culture or antibiogram as basis of choice. This empirical therapy may increase antimicrobial resistance in bacterial pathogens and have a negative impact on remaining skin commensals. The restoration of bacterial homeostasis is considered substantial in wound healing and the discussion on the propriety and management of antibacterial treatment and alternative treatment options is ongoing.<sup>20,33</sup>

Although there are clear indications in our data regarding this issue, the cross-sectional field study design is certainly the main limitation. The treatment schedule before the sampling was out of our control: for example, different antibacterial agents were used. Although the size of our study population (80 horses) can be regarded as a solid base, it is too small to allow for further inferences into treatment type. Altogether, further longitudinal studies with defined treatment regimens are needed to resolve more detailed questions.

The analysis of intra- and interindividual differences between control pasterns substantiated our chosen sampling scheme. Here, we could show that different control pasterns of the same horse share a more similar microbiota than pasterns of different horses or even pasterns of horses from different stables. Thus, it may be suggested that alterations in affected pasterns are best studied in comparison to control pasterns of the same horse, and this also should be considered in subsequent studies.

In conclusion, our study highlights the association of EPD and the pastern skin microbiota. Severe manifestations of EPD involve not only a reduced bacterial diversity, but also a profound disordering of the microbiota with the potential of some species to dominate others. Also, although antibacterial treatment may not be the decisive factor for overall diversity reduction, our observations indicate that its benefit might be questionable and that

additional clinical studies are needed to establish the role of bacteria in the pathogenesis of EPD.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** The supplementary material features further information on the recruitment of participants, the utilized EPD scoring system, the study population, the investigated samples, as well as additional analyses based on unweighted Bray-Curtis dissimilarity indices

## Résumé

**Contexte** – La dermatite des paturons équine (EPD) est un syndrome multifactoriel qui se manifeste par des lésions cutanées de sévérité variable du paturon. Malgré l'utilisation répandue d'antibiotiques pour le traitement, on en sait peu sur le rôle des bactéries.

**Hypothèses/Objectifs** – Etudier le microbiote bactérien cutané des paturons atteints de EPD et de contrôles sains.

**Sujets** – Une étude contrôlée avec 80 chevaux de propriétaires ; chacun avec au moins un paturon atteint d'EPD et un paturon sain.

**Matériel et méthode** – Les chevaux ont été groupés selon la forme d'EPD (modérée, exsudative ou proliférative) le grade de sévérité et le type de prétraitement (désinfectant, antibiotique topique ou pas d'antibactérien). Des écouvillons cutanés ont été obtenus et la composition du microbiote a été comparée entre les groupes.

**Résultats** – La diversité bactérienne alpha était réduite sur les paturons atteints ( $P < 0.001$ ) et cette réduction était significativement associée à la forme de l'EPD ( $P < 0.001$ ) et pas avec le type de prétraitement ( $P > 0.14$ ). Les analyses de diversité bêta ont confirmé un désordre du microbiote cutané ( $P = 0.004$ ) des paturons atteints versus contrôles, ce qui était particulièrement marqué pour les lésions les plus sévères. Le type de prétraitement n'était pas significativement associé avec ces altérations. Quatre familles différentes sont été détectées parmi lesquelles, Staphylococcaceae était la plus importante. La relative abondance de staphylococci était significativement augmentée sur les paturons atteints ( $P = 0.011$ ), en particulier chez ceux qui ont reçu un prétraitement antibactérien.

**Conclusions et importance clinique** – Les changements du microbiote sont associés avec la forme d'EPD ou la sévérité des lésions. Le rôle des bactéries dans la pathogénie de l'EPD aussi bien que les propriétés et les conséquences d'un traitement antibactérien, devraient ainsi être plus étudiés.



## RESUMEN

**Introducción** – la dermatitis de la cuartilla equina (EPD), un síndrome multifactorial, se manifiesta como lesiones cutáneas de gravedad variable en el área de la cuartilla. A pesar del uso generalizado de la terapia antibacteriana para tratar esta afección, se sabe poco acerca de las bacterias contribuyentes.

**Hipótesis/Objetivos** – Investigar la microbiota bacteriana de la piel en metacarpos afectados y no afectados (control) por EPD.

**Animales** – estudio de casos y controles con 80 caballos de propietarios particulares; cada uno con al menos una cuartilla afectada por EPD y una cuartilla de control.

**Métodos y materiales** – los caballos se agruparon según la forma de EPD (leve, exudativa o proliferativa), el grado de gravedad asignado y el tipo de pretratamiento (desinfectante, antibacteriano tópico o sin pretratamiento antibacteriano). Se obtuvieron hisopos de piel y se comparó la composición de la microbiota entre los grupos.

**Resultados** – la diversidad alfa bacteriana se redujo en los metacarpos afectados ( $P < 0,001$ ) y esta reducción se asoció significativamente con las formas de EPD ( $P < 0,001$ ) y no con el tipo de pretratamiento ( $P > 0,14$ ). Los análisis de la diversidad beta confirmaron un trastorno de la microbiota de la piel ( $P = 0,004$ ) en los metacarpos afectados frente al control, que fue particularmente intenso en las lesiones más graves. El tipo de pretratamiento no se asoció significativamente con este trastorno. Se detectaron cuatro familias diferencialmente abundantes, de las cuales Staphylococcaceae fue la más distintiva. La abundancia relativa de estafilococos aumentó significativamente en los metacarpos afectados ( $P = 0,011$ ), particularmente en aquellos que habían recibido tratamiento antibacteriano previamente.

**Conclusiones y relevancia clínica** – los cambios en la microbiota están asociados con la forma de EPD o la gravedad de las lesiones. Por lo tanto, el papel de las bacterias en la patogenia de la EPD, así como la propiedad y las consecuencias del tratamiento antibacteriano, deben investigarse más a fondo.

## Zusammenfassung

**Hintergrund** – Die equine Pastern Dermatitis (Mauke), ein multifaktorielles Syndrom, manifestiert sich in Form von Hautveränderungen von unterschiedlichem Ausmaß in der Fesselbeuge. Trotz der weitverbreiteten Verwendung antibakterieller Therapie zur Behandlung dieses Zustandes, ist wenig bekannt über die beteiligten Bakterien.

**Hypothese/Ziele** – Eine Untersuchung der bakteriellen Mikrobiota der Haut bei EPD-betroffenen und nicht-betroffenen (Kontroll) Fesselbeugen.

**Tiere** – Eine Fallkontrollstudie mit 80 Pferden in Privatbesitz; ein jedes mit mindestens einer EPD-betroffenen und einer Kontroll-Fesselbeuge.

**Methoden und Materialien** – Die Pferde wurden nach der Form der EPD in Gruppen eingeteilt (mild, exsudativ oder proliferativ), dem zugeteilten Schweregrad und der Art der Vorbehandlung (Desinfektionsmittel, oberflächliche antibakterielle oder nicht-antibakterielle Vorbehandlung). Es wurden Hauttupfer genommen und die Zusammensetzung der Mikrobiota zwischen den Gruppen verglichen.

**Ergebnisse** – Die bakterielle Alpha Diversität war in den betroffenen Fesselbeugen reduziert ( $P < 0,001$ ) und diese Reduzierung stand signifikant im Zusammenhang mit den EPD Formen ( $P < 0,001$ ) und nicht mit der Art der Vorbehandlung ( $P > 0,14$ ). Die Analyse der Beta-Diversität bestätigte ein Durcheinander der Mikrobiota der Haut ( $P = 0,004$ ) in betroffenen versus den Kontroll Fesselbeugen, was in manchen schweren Fällen besonders auffällig war. Die Art der Vorbehandlung stand nicht signifikant im Zusammenhang mit diesem Durcheinander. Es wurden vier verschiedenen häufig auftretende Familien gefunden, von denen Staphylokokken die häufigsten waren. Die relative Häufigkeit der Staphylokokken war in den betroffenen Fesselbeugen signifikant erhöht ( $P = 0,011$ ), vor allem bei denen, die vorher bereits eine antibakterielle Behandlung erhalten hatten.

**Schlussfolgerungen und klinische Bedeutung** – Veränderungen der Mikrobiota standen im Zusammenhang mit der EPD Form oder der Schwere der Veränderungen. Die Rolle der Bakterien bei der Pathogenese der EPD sowie die Korrektheit und die Konsequenzen der antibakteriellen Behandlung sollten daher in Zukunft noch untersucht werden.

## 要約

**背景** – 多因子症候群の一つである馬の繋轡 (EPD) は、繋轡部に様々な重症度の皮膚病変を現す。馬の繋轡治療には抗菌療法が広く用いられているが、原因となる細菌についてはほとんど知られていない。

**仮説・目的** – 本研究の目的は、EPDに罹患および罹患していない (コントロール) 繋轡部における細菌性皮膚微生物叢を調査することであった。

**被験動物** – クライアント所有馬80頭を対象としたケースコントロール研究で、それぞれ少なくとも1頭のEPD罹患馬および1頭の対照馬を対象とした。

**材料と方法** – 馬はEPDの形態 (軽度、滲出性、増殖性)、割り当てられた重症度、前治療の種類 (消毒剤、局所抗菌剤、抗菌剤前処理なし) によってグループ分けされた。また、皮膚スワブを採取し、グループ間の微生物叢組成を比較した。

**結果** – 細菌のa多様性は罹患繋部で減少し ( $P < 0.001$ )、この減少はEPD形態と有意に関連し ( $P < 0.001$ )、前治療の種類とは関連しなかった ( $P > 0.14$ )。b多様性の解析では、患肢および対照肢で皮膚微生物叢の乱れ ( $P = 0.004$ ) が確認され、特に重度の病変で顕著であった。前治療の種類は、この乱れとは有意に関連していなかった。4つの異なるファミリーが検出され、中でもStaphylococcaceaeが最も特徴的であった。Staphylococciの相対的な存在量は、罹患繋部で有意に増加し ( $P = 0.011$ )、特に以前に抗菌治療を受けたものでは顕著であった。

**結論と臨床的関連性** – 微生物相の変化はEPDの形態または病変の重症度と関連している。したがって、EPDの病因における細菌の役割や、抗菌剤治療の妥当性および結果について、さらに調査する必要がある。

**背景** – 馬股皮炎(EPD)是一种多因素综合征,表现为不同严重程度的骹部皮肤病变。尽管抗菌治疗被广泛用于治疗这种疾病,但对相关细菌知之甚少。

**假设/目的** – 研究EPD的发病和未发病(对照) 骹的细菌皮肤微生物群。

**动物** – 病例对照研究, 80匹私家马; 每匹马至少有1处骹部发生和未发生EPD。

**方法和材料** – 马按EPD形式(轻度、渗出性或增生性)、指定的严重程度等级和前期治疗类型(消毒剂、外部抗菌或无抗菌治疗) 分组。留取皮肤拭子, 比较各组菌群组成。

**结果** – 发病骹的细菌a多样性降低( $P < 0.001$ ), 这种降低与EPD形式显著相关( $P < 0.001$ ), 而与治疗类型无关( $P > 0.14$ )。b多样性分析证实, 与对照骹相比, 发病骹的皮肤微生物群紊乱( $P = 0.004$ ), 在更严重的病变中尤其明显。治疗类型与这种紊乱无显著相关性。检测到丰度不同的4个科, 其中葡萄球菌科最明显。发病骹中葡萄球菌的相对丰度显著增加( $P = 0.011$ ), 特别是既往接受过抗菌治疗的骹。

**结论和临床相关性** – 微生物群的变化与EPD形式或病变严重程度相关。因此应进一步研究细菌在EPD发病机制中的作用, 以及抗菌治疗的适当性和后果。

## Resumo

**Contexto** – A dermatite de quartela equina (DQE), uma síndrome multifatorial, manifesta-se como lesões cutâneas de gravidade variável na região da quartela. Apesar do amplo uso de antibioticoterapia para o tratamento desta condição, pouco se sabe sobre as bactérias que contribuem.

**Hipótese/Objetivos** – Investigar a microbiota bacteriana quartelas DQE-afetadas e quartelas controle.

**Animais** – Estudo caso-controle com 80 cavalos de tutores; cada um com ao menos uma quartela DQE-afetada e uma quartela controle.

**Métodos e matérias** – Os cavalos foram agrupados de acordo com a sua forma de DQE (leve, exsudativa ou proliferativa), o grau de severidade atribuído e o tipo de pré-tratamento (desinfetante, antibacteriano tópico ou sem pré-tratamento antibacteriano). Obteve-se *swabs* cutâneos, e a composição da microbiota foi comparada entre os grupos.

**Resultados** – A alfa diversidade bacteriana estava reduzida nas quartelas afetadas ( $P < 0,001$ ) e esta redução foi significativamente associada com as formas de DQE ( $P < 0,001$ ), não ao tipo de pré-tratamento ( $P > 0,14$ ). As análises de beta diversidade confirmaram o distúrbio da microbiota cutânea ( $P = 0,004$ ) nas quartelas afetadas *versus* controle, que foi particularmente mais intensa nas lesões mais severas. O tipo de pré-tratamento não estava associado com esse distúrbio. Quatro famílias diferentemente abundantes foram encontradas, das quais a Staphylococcaceae foi a mais distinta. A abundância relativa de estafilococos estava significativamente aumentada nas quartelas afetadas ( $P = 0,011$ ), particularmente naquelas que receberam antibacterianos previamente.

**Conclusões e importância clínica** – As alterações na microbiota estão associadas à forma da DQE e à severidade das lesões. A participação das bactérias na patogênese da DQE e as propriedades e consequências do tratamento antibacteriano devem ser investigadas futuramente.